Association of exposure to manganese and iron with relaxation rates R1 and R2*– magnetic resonance imaging results from the WELDOX II study

Beate Pesch[a*], Ulrike Dydak[b,c*], Anne Lotz[a], Swaantje Casjens[a], Clara Quetscher[a], Martin Lehnert[a], Jessica Abramowski[a], Christoph Stewig[a], Chien-Lin Yeh[b,c], Tobias Weiss[a], Christoph van Thriel[d], Lennard Herrmann[e], Siegfried Muhlack[e], Dirk Woitalla[e], Benjamin Glaubitz[f], Tobias Schmidt-Wilcke[f,g], Thomas Brüning[a]

[a]Institute for Prevention and Occupational Medicine of the German Social Accident Insurance, Institute of the Ruhr-Universität Bochum (IPA), Bochum, Germany

[b]School of Health Sciences, Purdue University, West Lafayette, IN, USA

[c]Department of Radiology and Imaging Sciences, Indiana University School of Medicine, Indianapolis, IN, USA

[d]Leibniz Research Centre for Working Environment and Human Factors (IfADo), Dortmund, Germany

[e]Department of Neurology, Sankt Josef Hospital, Bochum, Germany

[f]Department of Neurology, BG University Hospital Bergmannsheil, Ruhr-Universität Bochum, Germany

[g]Department of Neurology, St. Mauritius Hospital Meerbusch, Institute of Clinical Neuroscience and Medical Psychology, University Hospital Düsseldorf, Meerbusch, Germany

*equally contributed

Corresponding author:

Beate Pesch, PhD

Institute for Prevention and Occupational Medicine of the German Social Accident Insurance, Institute of the Ruhr-Universität Bochum (IPA)

This is the author's manuscript of the article published in final edited form as:
Pesch, B., Dydak, U., Lotz, A., Casjens, S., Quetscher, C., Lehnert, M., … Brüning, T. (2017). Association of exposure to manganese and iron with relaxation rates R1 and R2*– magnetic resonance imaging results from the WELDOX II study. NeuroToxicology. https://doi.org/10.1016/j.neuro.2017.08.008

Phone: +49-234-302 4506, e-mail: pesch@ipa-dguv.de

Highlights

- Blood manganese was associated with the relaxation rate R1 in the globus pallidus.
- Serum ferritin was a predictor of the relaxation rate R1 in the globus pallidus.
- Former welders had no increased relaxation rate R1 compared to controls.
- Respirable Mn above 100 µg/m³ was associated with stronger R1 signals in welders.

Abstract

Objective

Magnetic resonance imaging is a non-invasive method that allows the indirect quantification of manganese (Mn) and iron (Fe) accumulation in the brain due to their paramagnetic features. The WELDOX II study aimed to explore the influence of airborne and systemic exposure to Mn and Fe on the brain deposition using the relaxation rates R1 and R2* as biomarkers of metal accumulation in regions of interest in 161 men, including active and former welders.

Material and methods

We obtained data on the relaxation rates R1 and R2* in regions that included structures within the globus pallidus (GP), substantia nigra (SN), and white matter of the frontal lobe (FL) of both hemispheres, as well as Mn in whole blood (MnB), and serum ferritin (SF). The study subjects, all male, included 48 active and 20 former welders, 41 patients with Parkinson's disease (PD), 13 patients with hemochromatosis (HC), and 39 controls. Respirable Mn and Fe were measured during a working shift for welders. Mixed regression models were applied to estimate the effects of MnB and SF on R1 and R2*. Furthermore, we estimated the influence of airborne Mn and Fe on the relaxation rates in active welders.

Results

MnB and SF were significant predictors of R1 but not of R2* in the GP, and were marginally associated with R1 in the SN (SF) and FL (MnB). Being a welder or suffering from PD or HC elicited no additional group effect on R1 or R2* beyond the effects of MnB and SF. In active welders, shift concentrations of respirable Mn >100 $\mu g/m^3$ were associated with stronger R1 signals in the GP. In addition to the effects of MnB and SF, the welding technique had no further influence on R1.

Conclusions

MnB and SF were significant predictors of R1 but not of R2*, indicative of metal accumulation, especially in the GP. Also, high airborne Mn concentration was associated with higher R1 signals in this brain region. The negative results obtained for being a welder or for the techniques with higher exposure to ultrafine particles when the blood-borne concentration was included into the models indicate that airborne exposure to Mn may act mainly through MnB.

Key words: Manganese; Ferritin; Magnetic resonance imaging; Relaxation rates; R1; R2*

1. Introduction

Occupational exposure to manganese (Mn) has been associated with neurotoxic effects along a continuum of dysfunctions ranging from subclinical neurobehavioral effects associated with low Mn exposure to manganism following high exposure (Martin 2006; Mergler et al. 1999). Expert panels, such as the German MAK Commission and the American Conference of Governmental Industrial Hygienists (ACGIH) have recommended occupational exposure limits (OELs) for respirable Mn as low as 20 $\mu g/m^3$ (Deveau et al. 2016), and consequently, the German OEL was set to this value in 2015. Compliance with this OEL is challenging for welders, in particular when applying high-emission techniques such as gas metal arc welding (GMAW) (Hobson et al. 2011). Given the low aerodynamic diameter of particles in welding fumes, most airborne Mn is respirable (Harris et al. 2005; Pesch et al. 2012). The content of ultrafine particles is especially high in tungsten inert gas welding (TIG) (Lehnert et al. 2012).

To prevent neurotoxic effects of Mn, OELs should be sufficiently low to avoid brain accumulation of Mn in occupationally exposed workers. In addition, iron (Fe), a major constituent of welding fumes that is strongly correlated with airborne Mn (Pesch et al. 2012), plays a prominent role in neurodegeneration (Ward et al. 2014). Although both redox-active metals are essential for the development and maintenance of normal brain functions, excess concentrations as a result of occupational exposure, aging or disease can induce oxidative damage (Bouabid et al. 2015; Zecca et al. 2004b). An accumulation of Mn in the globus pallidus (GP) has been associated with manganism, whereas, the accumulation of redox-active Fe has been observed in the substantia nigra (SN) of patients with Parkinson's disease (PD) (Fasano et al. 2006; Ward et al. 2014; Zecca et al. 2008; Zucca et al. 2015). In the context of magnetic resonance imaging (MRI),

paramagnetic metals enhance the relaxation rates R1 and R2* in affected regions of the brain (Fitsanakis et al. 2006). Thus, R1 and R2* have become growingly used biomarkers of Mn and Fe accumulation.

Due to their structural similarity, Mn and Fe compete for the same biological transport systems (Fitsanakis et al. 2010). This is reflected in higher blood concentrations of Mn (MnB) in subjects with low iron stores in the general population (Kim and Lee 2011; Meltzer et al. 2010; Oulhote et al. 2014). Bioavailable Mn and Fe are transported through the capillary endothelium of the blood-brain barrier, and can also enter the brain via the cerebrospinal fluid (Bouabid et al. 2015; Yokel 2006). Recent studies have shown that welders have higher blood concentrations of both metals compared to controls, and present with increased R1 and R2* in vulnerable brain regions (Lee et al. 2015; Lee et al. 2016). Whether airborne Mn or Fe can enter these brain regions by direct influx through the olfactory pathway remains to be investigated. Higher R1 values were also observed in neuroimaging studies with subjects suffering from other conditions leading to elevated MnB, such as liver cirrhosis or abuse of Mn-contaminated ephedrone (Maffeo et al. 2013; Poniatowska et al. 2014). Whereas the association of brain iron with R2* has been increasingly explored (Haacke et al. 2005b), less is known about the influence of the systemic iron load in terms of serum ferritin (SF) on brain iron levels or R2*. Patients with hemochromatosis (HC) are especially of interest as they may present with either increased SF if untreated, or with very low SF when regular phlebotomy is applied. Decreased R2* values in iron-rich regions, such as the SN were observed in two HC patients (Haba-Rubio et al. 2005).

In addition to occupational exposure limits for airborne exposure to Mn, a biological reference value of 15 µg/L has been set for MnB in Germany. This threshold value is the presumed 95th percentile of the distribution in the general population and higher concentrations are thought to be mainly caused by occupational exposure (Bonberg et al. 2017). However, no regulation has been issued to control airborne Fe or biomarkers of high iron stores in workers exposed to metal

fumes. Welders can present with high SF in addition to elevated MnB (Casjens et al. 2014). Concentrations of SF above 1000 µg/L are predictive of developing liver cirrhosis, for example in untreated HC patients (Wang et al. 2010). In addition, patients with liver cirrhosis showed increased MnB and Mn in the basal ganglia due to impaired biliary excretion (Krieger et al. 1995). High concentrations of the liver enzyme gamma-glytamyl transpeptidase (GGT) was significantly associated with elevated SF in welders (Casjens et al. 2014). This makes the interpretation of SF above normal values difficult in the context of lifestyle (e. g. alcohol consumption) and exposure to welding fumes.

Here, we present the MRI results from the WELDOX II study encompassing 161 men with and without exposure to welding fumes in order to explore the associations between airborne and systemic exposure to Mn and Fe with possible accumulation of these metals in specific brain regions using the relaxation rates R1 and R2* as MRI-based biomarkers. We included active and former welders as well as controls and enrolled PD and HC patients in order to increase the contrast in systemic or brain iron. We adjusted the relaxation rates acquired in both hemispheres for potential liver dysfunction and other covariates.

2. Material and methods

2.1. Study groups

Forty-eight male welders - not wearing a powered air-purifying respirator - from 14 companies were recruited between 2013 and 2015. Personal exposure to welding fumes was measured in the breathing zone during a working shift. Major techniques were gas metal arc welding (GMAW) (n=22) and tungsten inert gas welding (TIG) (n=19). A supplemental questionnaire documented the welding history and included information on welding process, consumable electrode, base material, respiratory protection, and other factors. Photos of the workplaces were used to document the efficacy of the local exhaust ventilation. In order to

determine MnB after the shift, blood samples were drawn and transported in a cooling box to the laboratory. The neuroimaging of the welders was performed with a median lag time of six weeks after this investigation.

We further enrolled 20 former welders with a median time of seven years since their last exposure to welding fume and 39 male controls from the general population of the greater Bochum area. Controls were eligible if they never worked as a welder and did not suffer from PD. Forty-one male patients with a confirmed diagnosis of PD (DW, SM, LH) who were under treatment from the Department of Neurology, Saint Joseph Hospital in Bochum were included in the study (median Hoehn-Yahr score: 2, median UPDRS3 score: 33). Thirteen male HC patients were recruited through a self-help organisation. All except one were treated by phlebotomy and did not suffer from liver cirrhosis.

A questionnaire was used to assess socio-demographic characteristics, occupational history, medications and chronic diseases in a face-to-face interview. All participants underwent a neurological assessment of movement disorders according to UPDRS3, which was video documented for an independent evaluation by a second reading, as well as additional examinations by experienced neurologists (DW, SM, LH). Participants also gave a blood sample to determine MnB (except active welders), blood count, iron metabolism, including SF and free serum iron (SFe), GGT and other liver enzymes on the day of neuroimaging at the Institute of Clinical Chemistry, Transfusion and Laboratory Medicine of the Berufsgenossenschaftliches Universitätsklinikum Bergmannsheil with methods formerly described for the WELDOX study (Casjens et al. 2014). SF was measured by ADVIA Centaur (Siemens, Eschborn, Germany) using a chemiluminometric sandwich immunoassay, standardized to WHO standard 80/578. Elevated GGT was considered an indicator of potential liver dysfunction, and carbohydrate-deficient transferrin (CDT) > 2.6% was presumptive for possible alcohol abuse.

All participants provided written informed consent prior to participation. The study protocol was approved by the Ethics Committee of the Ruhr University Bochum, Germany.

2.2. Airborne exposure to Mn and Fe

The concentrations of respirable Mn and Fe were determined as formerly described (Pesch et al. 2012). In brief, welders were equipped with PGP-EA samplers for collecting respirable particles at a flow rate of 3.5 L/min in the breathing zone (Lehnert et al. 2012). The average duration of the sampling was four hours. Mn and Fe were determined by inductively coupled plasma mass spectrometry (ICP-MS) with a Perkin Elmer Elan DRC II (Waltham, Massachusetts). One measurement of Mn and four measurements of Fe were below the limits of quantitation (LOQs) and converted to 0.5 * LOQ for statistical analyses.

2.3. Determination of manganese in whole blood

MnB was quantified by means of ICP-MS. To prevent contamination, only plastic materials were used for sample preparation. A reagent blank was included in each analytical series. Archived frozen blood samples were thawed and brought to room temperature. Then, 400 µL of whole blood were diluted 1:12.5 with a 0.5% solution of ammonium hydroxide and 100 µL of a 0.2% solution of Triton-X. Rhodium was used as the internal standard. Analysis was carried out using a 7700 ICP-MS system from Agilent Technologies (Waldbronn, Germany) in He-mode (flow rate: 5 mL/min) with a collision cell to avoid interferences. Skimmer and sampler cones were made of platinum. Calibration and calculation of the analytical result were carried out using standards prepared in sheep blood at eight different concentrations. All measurements were above the LOQ of 2.0 µg/L. For internal quality control, we used commercially available material from RECIPE (ClinChek Whole Blood Level, lyophil. for Trace Elements I and II, REF 8840, LOT 227) and SERONORM (Trace Elements Whole Blood Level I and II, LOT

1103129). Within-series imprecision and between-day imprecision were both lower than 8%. The accuracy of analytical results was ensured by successful participation in an international quality assessment scheme for analyses in biological materials.

2.4. MR image acquisition and analysis

MRI scans were performed on a 3 T Philips Achieva X-series whole-body clinical scanner (Philips Healthcare, Best, The Netherlands) with a 32-channel head coil. T1-weighted 3D turbo field echo images (T1 TFE, TR/TE=8.4/3.9 ms, flip angle=8°, bandwidth=191 Hz/pixel, 220 slices, voxel size $(1\ mm)^3$ isotropic, acquisition matrix: 240 x 240 mm, SENSE factor 2.5) were acquired for anatomical reference. Two fast field echo images (T1 FFE, TR/TE=8.4/3.7 ms, flip angles=3° and 17°, band width=191 Hz/pixel, 160 slices, voxel size $(1\ mm)^3$ isotropic, acquisition matrix: 256 x 256 mm, SENSE factor 2) were acquired for quantitative estimation of R1 (=1/T1) in regions of interest (ROIs). T1 values were calculated by the variable flip angle method (Sabati and Maudsley 2013). Dual-TR B1 maps were acquired to correct T1 maps for field inhomogeneity. Five 3D fast field echo images with different echo times (FFE, TR/TE/delta_TE=24.3/3.7/4.4 ms, flip angle=20°, bandwidth=287 Hz/pixel, 80 slices, voxel size $(1.5\ mm)^3$ isotropic, acquisition matrix: 256 x 256 mm, SENSE factor 2) were acquired to estimate R2* (=1/T2*) in the corresponding ROIs. T2* values were calculated by fitting the signal intensity as a function of echo time using the exponential decay model (Long et al. 2014). The ROI was measured from one single slice in each case. The R1 and R2* values for each ROI were calculated as 1/T1 and 1/T2*, respectively, as the median across each ROI, separately for the left and right hemisphere. ROIs were selected within the GP, SN and the white matter of the frontal lobe (FL) of both hemispheres (Figure 1). The placement of the ROI in the white matter of FL was motivated by changes in R1 observed in primates (Guilarte et al. 2006). We performed the positioning in duplicate and used the arithmetic means of R1 and R2* from both readers for the statistical analysis.

2.5. Statistics

Median and inter-quartile range (IQR) were used to describe the distribution of continuous variables in all men or subgroups. As R1 and R2* were acquired in both hemispheres, we presented their arithmetic mean for descriptive purposes but analysed the side-specific measurements of the relaxation rates together with the hemisphere as the covariate in the regression models. Welders were additionally stratified according to technique (GMAW, TIG and others), exposure to respirable Mn (low: <20 µg/m^3, medium: 20-100 µg/m^3, high: >100 µg/m^3) or Fe (low: <25 µg/m^3, medium: 25-310 µg/m^3, high: >310 µg/m^3). Spearman correlation coefficients (r_s) between airborne, systemic and brain exposure to Mn and Fe were shown with their 95% confidence interval (CI). We applied linear mixed models to explore the association of R1 and R2*, respectively, with the study group, and MnB and SF as exposure variables. The subjects were implemented as a random factor to consider the dependence of the measurements on the left and right hemisphere. We further ran mixed models to investigate the association of R1 and R2* with respirable Mn and Fe in active welders. All models were adjusted for age, GGT, and hemisphere. We additionally implemented R2* in the modeling of R1 and *vice versa*. The calculations were performed with the statistical software SAS, version 9.4 (SAS Institute Inc., Cary, NC, USA).

3. Results

3.1. Characteristics of the study groups

Table 1 shows the distribution of the socio-demographic characteristics in all men and in the respective study groups. Active welders were younger than former welders (median age 51 and 65 years, respectively). Former welders had a median value of seven years since their last exposure to welding fumes (data not shown). Patients with PD had an average age of 60 years, and those with HC were on average 56 years of age. Controls were recruited whose ages were within the range of those in the different study groups, with a median age of 54 years. Nearly every other active welder was a current smoker, but none of the HC

patients was currently smoking. All welders had a blue-collar job as their longest held occupation; whereas, the proportion of blue-collar jobs was 41% in the controls, 49% in PD, and 23.1% in HC. Elevated GGT (≥56 U/L) was more frequently observed in welders (35.0% in former welders, 18.8% in active welders) than in the other participants (15% in controls and PD patients, 7.7% in HC). Eight men (including six welders) presented with CDT >2.6%.

3.2. Distribution of exposure to Mn

We assessed exposure to Mn using the shift concentration of respirable Mn in active welders, the systemic burden with MnB, and used R1 as a biomarker of brain accumulation in all men. **Table 2** shows the medians with IQRs of the distributions of MnB and R1 in the study groups. MnB was highest in active welders (7.7 µg/L) and lowest in HC patients (6.2 µg/L), with the medians of the other groups ranging between 6.4 and 6.6 µg/L. Two active welders and one HC patient had MnB >15 µg/L (data not shown). We observed a corresponding pattern for R1 in the GP, with the highest median in active welders (0.87 1/sec) and the lowest in HC (0.81 1/sec). HC patients also showed the lowest median of R1 in the SN; whereas, active welders were within the range of the other groups. Notably, former welders did not present with higher R1 values compared to the controls in GP and SN.

Table 3 presents the distribution of respirable Mn and R1 in welders stratified by GMAW as the high-emission technique (22 active and 13 former welders) *versus* TIG and other processes (26 active welders and seven former welders). The median shift exposure to respirable Mn was 68 (IQR 33-140) µg/m^3 in GMAW, but 5 (IQR 2-23) µg/m^3 in TIG and other techniques. The median MnB was 9.2 µg/L in active and 6.3 µg/L in former GMAW welders. The corresponding medians were 7.3 µg/L in active and 6.7 µg/L in former welders using TIG or techniques other than GMAW. The medians of R1 were similar in both groups among active welders in all ROIs (e.g. GP 0.87, SN 0.76). Active GMAW welders had slightly higher medians of R1 in the GP and FL than former GMAW welders.

3.3. Distribution of exposure to Fe

We assessed exposure to Fe with the shift concentration of respirable Fe in active welders, free (SFe) and ferritin-bound Fe (SF) as serum markers and used R2* as a biomarker of brain iron accumulation in all men. **Table 2** shows the distributions of SFe, SF and R2* in the study groups with median and IQR values. SFe was highest in HC (126 µg/dL) and lowest in former welders and PD patients (94 µg/dL and 95 µg/dL, respectively); whereas, an opposite pattern was observed for SF (45 µg/L in HC, 113 µg/L in PD, 242 µg/L in former welders). Active welders had SFe concentrations that were similar to controls (103 µg/dL and 105 µg/dL), but their median SF levels were higher (129 µg/L and 74 µg/L, respectively). Active welders did not exhibit stronger R2* signals compared to controls or former welders in any of the ROIs. Notably, PD patients presented with slightly higher medians of R2* in their GP and SN than those with HC or the other groups.

Table 3 depicts the distribution of the exposure variables with regard to Fe among welders. The median shift exposure to respirable Fe was 370 (IQR 190-760) µg/m^3 in GMAW, and 15 (IQR 5-84) µg/m^3 in TIG and other techniques. SF but not SFe showed a corresponding pattern in active welders, but with less pronounced differences than the airborne concentrations (GMAW: 152 µg/L; TIG and other techniques: 108 µg/L). Former welders who used major techniques other than GMAW presented with very high SF (median 460 µg/L). Medians of R2* of all ROIs were quite similar in these subgroups among active or former welders.

3.4. Associations between the exposure variables

In **Table 4,** we present Spearman rank correlation coefficients with 95% CIs showing the associations between exposure variables in all participants. Correlations with airborne Mn and Fe were shown for active welders. Respirable Mn and Fe strongly correlated in welding fumes (r_s 0.93, 95% CI 0.88-0.96). Both metals

correlated with SF (Mn: r_s 0.32, 95% CI 0.04-0.56; Fe: r_s 0.36, 95% CI 0.09-0.59), but not with MnB. In all participants, we observed a negative correlation between MnB and SFe. Whereas we found indications of weak associations of MnB with R1 (FL: r_s 0.18, 95% CI 0.02-0.33), the correlations between systemic iron markers and R1 were less clear. None of the shift measurements of respirable Mn or Fe correlated with R1 or R2*. Also, no significant associations were observed between MnB, SF or SFe with any of the R2* measurements. We found moderate correlations between the R1 values of the three ROIs and a strong association of the R2* measurements in the GP and SN (r_s 0.61, 95% CI 0.50-0.70). With regard to FL, we observed a weak correlation between R1 and R2* (r_s 0.23, 95% CI 0.07-0.38).

3.5. Potential predictors of the relaxation rate R1 in ROIs

Table 5 presents the effects estimates of potential determinants of R1 adjusted for covariates. MnB was a significant predictor of R1 in the GP (p=0.004), and showed a marginal influence on R1 in the FL (p=0.06). SF was associated with R1 in the GP (p=0.005) and SN (p=0.04). We found no additional effect of being an active welder or PD patient in comparison to the controls. HC patients presented with marginally lower R1 values in all ROIs, but this observation was based on small numbers (FL: p=0.07). Former welders had even slightly lower R1 relaxation times in these ROIs compared to the controls, an observation that was also not significant (GP: p=0.08). Furthermore, a higher attained age resulted in lower R1 in the GP (p=0.037) and FL (p<0.001). Furthermore, R1 was associated with R2* in the GP (p=0.025). We found no association of GGT with the R1 values in these ROIs. The level-two variance estimates of the mixed models reflect the correlations between the R1 values that were acquired in the respective ROIs of both hemispheres.

When restricting this model to active and former welders, MnB and SF remained significant predictors of R1, especially in the GP, but the major welding technique (GMAW *versus* TIG and others) did not additionally influence R1 (**Table 6a**). **Table 6b** depicts the effect estimates for airborne Mn on R1 in active

welders. We observed higher R1 values in the GP when welders were exposed to respirable Mn above 100 µg/m^3 (p=0.01). Again, there was no association of GGT with R1. When airborne Mn was substituted by respirable Fe, the R1 signals in the GP were not significantly elevated in welders (data not shown).

*3.6. Potential predictors of the relaxation rate R2**

The data in **Table 5** also demonstrate the effect estimates for potential predictors of R2* in the GP, SN and FL. Neither SF nor MnB were significant determinants of R2*. When compared to the controls, welders and patients with PD or HC had slightly lower R2* values in their GP, although not significant. Former welders and PD patients had somewhat stronger R2* values in the SN, although statistically not significant. Furthermore, a higher attained age resulted in an increase of R2* in the GP (p=0.032) and SN (p=0.006). R1 was associated with R2* in the GP (p=0.03) and the FL (p=0.018). The level-two variance estimates of the mixed models reflect the correlations between R2* values of the corresponding ROIs of both hemispheres.

As shown in **Table 6b,** we observed no association between the R2* values and the concentrations of respirable Fe in active welders. Welders assigned to the highest tertile of airborne Fe (>310 µg/m^3) presented with slightly lower values in the GP and SN as compared to low-exposed welders (<25 µg/m^3), although not significant.

4. Discussion

Mn is essential to steel production to improve the workability and hardness of steel, and welding is one of the primary industrial activities to process steel. Based on the available evidence that Mn, even at low concentrations, may cause neurotoxic effects, occupational exposure limits were reduced in Germany and other countries (Bevan et al. 2017). The majority of GMAW welders in this and the previous WELDOX study were exposed to concentrations of respirable Mn above

the current German OEL of 20 µg/m^3 (Pesch et al. 2012; Lehnert et al. 2014). An important prerequisite necessary to prevent neurotoxicity is the avoidance of brain accumulation of Mn in welders and other at-risk occupations. However, Fe is the major constituent of welding fumes, and welders can present with high SF under certain exposure circumstances (Casjens et al. 2014). Although there is mounting evidence that iron metabolism in the brain is implicated in neurodegeneration (Rouault 2013), there is yet limited evidence associating the iron metabolism in the blood and brain. Here, we analyzed the association of measures of exposure to Mn and Fe with the MRI relaxation rates R1 and R2* as biomarkers for metal accumulation in vulnerable brain regions, such as the GP (Lucchini et al. 2000). MnB and SF were found to be significant predictors of R1 in the GP in all men as well as in welders only, with some indication of accumulation in other ROIs, including areas within the SN and the white matter of the frontal lobe.

Our statistical models support the hypothesis that exposure to Mn might act via MnB on R1 with the blood-brain barrier as the major path of entry into these regions. Subjects with inhalational uptake of Mn, here active welders and in particular TIG welders with exposure to more ultrafine particles, did not show higher R1 values in these ROIs when adjusted for MnB and SF. A shift concentration of respirable Mn above 100 µg/m^3 was associated with stronger R1 signals in the GP compared with concentrations below the current German OEL of 20 µg/m^3 for this particle size fraction. Whether airborne Mn acts through elevated MnB on R1 or can also enter the brain via olfaction remains to be investigated.

Slightly lower R1 values in former welders who participated in our neuroimaging study support the notion that occupational exposure to Mn does result in a persistent Mn accumulation in the brain. Recovery from Mn-induced R1 signals after cessation of exposure to welding fumes has been observed in welders, as well as in experimental animals (Han et al. 2008; Han et al. 2014). However, cross-sectional studies are less informative than longitudinal intervention studies on the time course of an accumulation and clearance of Mn in selected brain regions. The limitations of this study design may explain conflicting results on the effects of long-term or short-term Mn exposure (Choi et al. 2007, Criswell et al. 2012, Lee et al. 2015).

Increased concentrations of MnB have been frequently observed in welders (Ellingsen et al. 2006; Long et al. 2014) and other at-risk occupations (Cowan et al. 2009; Myers et al. 2003). This finding was also supported in WELDOX II, where active welders had a higher median MnB than other participants. Furthermore, the higher concentrations of respirable Mn when using high-emissions techniques such as GMAW in comparison to TIG were reflected in higher MnB as already shown in the WELDOX study where we observed a potential overload of the systemic homeostasis of MnB when Mn exceeded 50-100 µg/m^3 (Pesch et al. 2012). This is in line with stronger R1 signals in the GP of welders with respirable Mn above 100 µg/m^3 and with the observation of a non-linear brain accumulation of Mn in other studies (Lee et al. 2015). Based on published as well as their own data, Baker and coworkers found a non-linear association between airborne Mn and MnB and concluded that concentrations of airborne Mn above 10 µg/m^3 already result in elevated MnB (Baker et al. 2014). However, various differences between these studies, mainly in the sampling of particulate matter in the breathing zone of workers, may impair a sound conclusion on the point of departure with regard to the concentration of airborne Mn, which may cause a deviation from the homeostatic regulation of MnB.

Whereas associations of MnB with R1 have been extensively studied, less is known about the significant association between SF and R1, which we could demonstrate as a novel finding in the present study. SF can serve as a biomarker of body iron stores, but is additionally affected by inflammation and liver dysfunction (Wang et al. 2010). We observed an influence of respirable Fe on SF in the current, as well as in the previous WELDOX study (Casjens et al. 2014; Pesch et al. 2012). Mn and Fe are strongly correlated in welding fumes, and their effects on neuroimaging or neurotoxic outcomes cannot be disentangled with statistical methods. However, MnB and SF showed only a negligible correlation due to the tight biological regulation of both redox-active metals, which allows a better assessment of the effects of the parent metals, Mn and Fe. We also found no associations between welding at high airborne Fe or elevated SF with R2* in

the GP, in agreement with a recent study in US welders (Lee et al. 2016). Here, the authors found higher R2* values in the caudate nucleus of welders, an iron-rich structure, which is highly innervated by dopaminergic neurons that originate from the SN. They also observed a correlation of whole-blood Fe on R2* in this region. This finding indicates the accumulation of Fe in vulnerable brain regions of welders, and illustrates the need for more research to explore the association between iron metabolism in blood and brain.

Accumulation of iron in the SN and other regions of the brain could be demonstrated in patients with PD and other neurodegenerative diseases with elevated R2* or post-mortem tissue analyses (Haacke et al. 2005). In the brain, Fe is also incorporated into ferritin in order to protect against oxidative damage. Ferritin is part of the neuromelanin complex, which is highly concentrated in the SN (Zecca et al. 2008). R2* measurements in the SN ranked highest in PD patients who are known to show a progressive loss of neuromelanin with the release of Fe (Ayton and Lei 2014; Faucheux et al. 2003). In addition, former welders who presented with the highest median SF among all groups showed somewhat higher R2* values in the SN, although this observation was based on small numbers with limited statistical power. We further observed an age-related increase in the R1 and R2* measurements in particular in the SN, which may reflect a general release of Fe from the neuromelatonin complex during aging (Zecca et al. 2004a).

So far, only a few studies subjected HC patients to neuroimaging in order to determine R1 or R2*, although this is one of the most commonly inherited genetic diseases that results in iron overload and liver cirrhosis if untreated (Powell et al. 2016). Most patients of our study were treated with regular phlebotomy, which may explain the low concentrations of SF (median 45 µg/L) compared to an average of around 150 µg/L in adult men (Pan and Jackson 2008). A similarly low SF concentration was observed in a male HC patient who was treated with phlebotomy and showed decreased R2* values in iron-rich brain regions (Haba-Rubio et al. 2005). In contrast to higher MnB in subjects with low SF in the general population (Oulhote et al. 2014), these HC patients presented with the lowest

median MnB among all study groups. Only one HC patient with a rare mutation had very high MnB. Low levels of both systemic markers, SF and MnB, may explain the lower medians of R1 and R2*, especially in the GP of HC patients compared to the other participants. The concentrations of free serum iron were however higher, although the observed effects on the relaxation rates were not significant due to the small number of patients.

High SF is indicative of liver dysfunction, predicts the risk of cirrhosis and is the main clinical manifestation of hemochromatosis (Wang et al. 2010; Powell et al. 2016). However, welders can also present with elevated SF (Pesch et al. 2012; Powell et al. 2016). In particular, nine out of 20 former welders in this study had SF above 300 µg/L. Furthermore, high concentrations of the liver enzyme GGT were associated with elevated SF in a larger group of active welders in the previous WELDOX study (Casjens et al. 2014). Furthermore, increased SF was also associated with elevated CDT in welders (Ellingsen et al. 2014). When CDT was implemented into the models that were applied to the results of the neurobehavioral tests, the effect that was formerly assigned to Mn could be partially explained by the higher alcohol consumption of welders compared with controls (Ellingsen et al. 2008; Ellingsen et al. 2013). Patients with liver cirrhosis have on average three times higher MnB than welders, and exhibit hyperintense R1 values (Krieger et al. 1995). However, we found no indication that GGT as marker of liver dysfunction influences R1 or R2*. In the present study, six welders were among the eight men with CDT >2.6%, which is commonly considered an indicator of alcohol abuse. The role of airborne Fe in iron metabolism in the blood and brain of welders, as well as the possible damage exerted to the liver due to chronically elevated SF need to be better explored, also in studies aimed to investigate the neurotoxicity of Mn. In welding fumes, Mn and Fe are strongly correlated. Hence, potential metal effects on the relaxations rates or on the results of neurobehavioral tests cannot be disentangled if the exposure assessment is based on air measurements only.

An advantage of this neuroimaging study is the relatively large study size facing the efforts to investigate volunteering subjects by MRI. The inclusion of different study groups such as HC patients enhanced the contrast in the variables under study, which is important for the confirmation of established and the identification of novel associations. On the other hand, the cross-sectional design is a limitation of this MRI study, as long-term exposure to these metals cannot be assessed with repeated measurements. Although we found relatively strong effects for SF and MnB, the statistical power was not sufficient to estimate significant effects in specific subgroups, for example in former welders. New methodological challenges arise when using R1 and R2*. Although relaxation rates are commonly used in practice and mostly limited to ROI analyses, they require multi-echo scans that are sensitive to subject motion and other problems. For example, the assessment of brain regions with cells that accumulate metals may depend on their exact anatomical brain location. Hence, the manual placing of ROIs is subject to the expertise of the observer, which we controlled using independent raters. Small regions like the SN are more difficult to capture than larger regions like the GP. Furthermore, a ROI-based analysis of R1 and R2* is limited to *a priori* selected brain regions of interest, which do not represent the metal accumulation throughout the whole brain. Yet, it is known that Mn and Fe can also deposit in other structures of the brain not investigated in our study, such as e.g. the caudate (Lee et al. 2016; Criswell et al. 2012; Long et al. 2014). Recent advances include the use of MRI techniques with higher sensitivity, such as quantitative susceptibility mapping (QSM) to investigate brain iron (e.g. Acosta-Cabronero et al. 2016).

5. Conclusion

MnB and SF were independent significant predictors of R1, which is indicative of metal accumulation, especially in the GP. However, none of the investigated exposure variables influenced R2* in the ROIs selected for investigation. A concentration of respirable Mn above 100 $\mu g/m^3$ was associated with stronger R1 signals in the GP. GMAW welders showed similar R1 values as welders using TIG or other welding techniques when adjusting for MnB or SF, although TIG welding generates more ultrafine particles. Our results on the association between blood and brain Mn and Fe metabolism support the hypothesis that the blood-

brain barrier is the major route of metal transport into the brain. The monitoring of SF and a reduction of high iron stores in active as well as former welders should be taken into account as preventive action.

Acknowledgements

The WELDOX II study was supported by a grant from the Employer's Liability Insurance Association for Wood and Metals (Berufsgenossenschaft Holz und Metall). The determination of metal concentrations in welding fumes was performed at the Institute for Occupational Safety and Health of the German Social Accident Insurance (IFA), Sankt Augustin, Germany. We thank Dr. Katrin Pitzke and Tobias Schwank for their support in presenting these analyses. We appreciate the scientific support of Burkhard Mädler from PHILIPS, Germany. UD and CLY were supported through the NIH grant R01ES020529.

References

Acosta-Cabronero J, Betts MJ, Cardenas-Blanco A, Yang S, Nestor PJ (2016) In vivo MRI mapping of brain iron deposition across the adult lifespan. J Neurosci 36: 364-374

Ayton S, Lei P (2014) Nigral iron elevation is an invariable feature of Parkinson's disease and is a sufficient cause of neurodegeneration. Biomed Res Int: 581256; doi: 10.1155/2014/581256

Baker MG, Simpson CD, Stover B, Sheppard L, Checkoway H, Racette BA, Seixas NS (2014) Blood manganese as an exposure biomarker: state of the evidence. J Occup Environ Hyg 11: 210-217

Bevan R, Ashdown L, McGough D, Huici-Montagud A, Levy L (2017) Setting evidence-based occupational exposure limits for manganese. Neurotoxicology 58: 238-248

Bonberg N, Pesch B, Ulrich N, Moebus S, Eisele L, Marr A, Arendt M, Jöckel KH, Brüning T, Weiss T (2017) The distribution of blood concentrations of lead (Pb), cadmium (Cd), chromium (Cr) and manganese (Mn) in residents of the German Ruhr area and its potential association with occupational exposure in metal industry and/or other risk factors. Int J Hyg Environ Health 220: 998-1005

Bouabid S, Tinakoua A, Lakhdar-Ghazal N, Benazzouz A (2015) Manganese Neurotoxicity: behavioral disorders associated with dysfunctions in the basal ganglia and neurochemical transmission. J Neurochem 136: 677-691

Casjens S, Henry J, Rihs HP, Lehnert M, Raulf-Heimsoth M, Welge P, Lotz A, Van Gelder R, Hahn JU, Stiegler H, Eisele L, Weiss T, Hartwig A, Bruning T, Pesch B (2014) Influence of welding fume on systemic iron status. Ann Occup Hyg 58: 1143-1154

Cowan DM, Fan Q, Zou Y, Shi X, Chen J, Aschner M, Rosenthal FS, Zheng W (2009) Manganese exposure among smelting workers: blood manganese-iron ratio as a novel tool for manganese exposure assessment. Biomarkers 14: 3-16

Criswell SR, Perlmutter JS, Huang JL, Golchin N, Flores HP, Hobson A, Aschner M, Erikson KM, Checkoway H, Racette BA (2012) Basal ganglia intensity indices and diffusion weighted imaging in manganese-exposed welders. Occup Environ Med 69: 437-443

Deveau M, Maier A, Krewski D (2017) Application of a framework for the selection of an appropriate occupational exposure limit for manganese. Neurotoxicology 58:249-256

Ellingsen DG, Chashchin M, Berlinger B, Konz T, Zibarev E, Aaseth J, Chashchin V, Thomassen Y (2014) Biomarkers of iron status and trace elements in welders. J Trace Elem Med Biol doi: 10.1016/j.jtemb.2014.03.004:

Ellingsen DG, Dubeikovskaya L, Dahl K, Chashchin M, Chashchin V, Zibarev E, Thomassen Y (2006) Air exposure assessment and biological monitoring of manganese and other major welding fume components in welders. J Environ Monit 8: 1078-1086

Ellingsen DG, Konstantinov R, Bast-Pettersen R, Merkurjeva L, Chashchin M, Thomassen Y, Chashchin V (2008) A neurobehavioral study of current and former welders exposed to manganese. Neurotoxicology 29: 48-59

Ellingsen DG, Kusraeva Z, Bast-Pettersen R, Zibarev E, Chashchin M, Thomassen Y, Chashchin V (2013) The interaction between manganese exposure and alcohol on neurobehavioral outcomes in welders. Neurotoxicol Teratol 41C: 8-15

Fasano M, Bergamasco B, Lopiano L (2006) Modifications of the iron-neuromelanin system in Parkinson's disease. J Neurochem 96: 909-916

Faucheux BA, Martin ME, Beaumont C, Hauw JJ, Agid Y, Hirsch EC (2003) Neuromelanin associated redox-active iron is increased in the substantia nigra of patients with Parkinson's disease. J Neurochem 86: 1142-1148

Fitsanakis VA, Zhang N, Avison MJ, Gore JC, Aschner JL, Aschner M (2006) The use of magnetic resonance imaging (MRI) in the study of manganese neurotoxicity. Neurotoxicology 27: 798-806

Fitsanakis VA, Zhang N, Garcia S, Aschner M (2010) Manganese (Mn) and iron (Fe): interdependency of transport and regulation. Neurotox Res 18: 124-131

Guilarte TR, McGlothan JL, Degaonkar M, Chen MK, Barker PB, Syversen T, Schneider JS (2006) Evidence for cortical dysfunction and widespread manganese accumulation in the nonhuman primate brain following chronic manganese exposure: a 1H-MRS and MRI study. Toxicol Sci 94: 351-358

Haacke EM, Cheng NY, House MJ, Liu Q, Neelavalli J, Ogg RJ, Khan A, Ayaz M, Kirsch W, Obenaus A (2005) Imaging iron stores in the brain using magnetic resonance imaging. Magn Reson Imaging 23: 1-25

Haba-Rubio J, Staner L, Petiau C, Erb G, Schunck T, Macher JP (2005) Restless legs syndrome and low brain iron levels in patients with haemochromatosis. J Neurol Neurosurg Psychiatry 76: 1009-1010

Han JH, Chung YH, Park JD, Kim CY, Yang SO, Khang HS, Cheong HK, Lee JS, Ha CS, Song CW, Kwon IH, Sung JH, Heo JD, Kim NY, Huang M, Cho MH, Yu IJ (2008) Recovery from welding-fume-exposure-induced MRI T1 signal intensities after cessation of welding-fume exposure in brains of cynomolgus monkeys. Inhal Toxicol 20: 1075-1083

Han SH, Ahn SW, Youn YC, Shin HW (2014) Reversal of pallidal magnetic resonance imaging T1 hyperintensity in a welder presenting as reversible parkinsonism. Neurol India 62: 117-118

Harris MK, Ewing WM, Longo W, DePasquale C, Mount MD, Hatfield R, Stapleton R (2005) Manganese exposures during shielded metal arc welding (SMAW) in an enclosed space. J Occup Environ Hyg 2: 375-382

Hobson A, Seixas N, Sterling D, Racette BA (2011) Estimation of particulate mass and manganese exposure levels among welders. Ann Occup Hyg 55: 113-125

Kim Y, Lee BK (2011) Iron deficiency increases blood manganese level in the Korean general population according to KNHANES 2008. Neurotoxicology 32: 247-254

Krieger D, Krieger S, Jansen O, Gass P, Theilmann L, Lichtnecker H (1995) Manganese and chronic hepatic encephalopathy. Lancet 346: 270-274

Lee EY, Flynn MR, Du G, Lewis MM, Fry R, Herring AH, Van BE, Van BS, Smeester L, Kong L, Yang Q, Mailman RB, Huang X (2015) T1 Relaxation Rate (R1) Indicates Nonlinear Mn Accumulation in Brain Tissue of Welders With Low-Level Exposure. Toxicol Sci 146: 281-289

Lee EY, Flynn MR, Du G, Li Y, Lewis MM, Herring AH, Van BE, Van BS, Kong L, Fry RC, Snyder AM, Connor JR, Yang QX, Mailman RB, Huang X (2016) Increased R2* in the Caudate Nucleus of Asymptomatic Welders. Toxicol Sci 150: 369-377

Lehnert M, Pesch B, Lotz A, Pelzer J, Kendzia B, Gawrych K, Heinze E, Van Gelder R., Punkenburg E, Weiss T, Mattenklott M, Hahn JU, Mohlmann C, Berges M, Hartwig A, Bruning T (2012) Exposure to inhalable, respirable, and ultrafine particles in welding fume. Ann Occup Hyg 56: 557-567

Lehnert M, Weiss T, Pesch B, Lotz A, Zilch-Schoneweis S, Heinze E, Van Gelder R, Hahn JU, Bruning T (2014) Reduction in welding fume and metal exposure of stainless steel welders: an example from the WELDOX study. Int Arch Occup Environ Health 87: 483-492

Long Z, Jiang YM, Li XR, Fadel W, Xu J, Yeh CL, Long LL, Luo HL, Harezlak J, Murdoch JB, Zheng W, Dydak U (2014) Vulnerability of welders to manganese exposure--a neuroimaging study. Neurotoxicology 45: 285-292

Lucchini R, Albini E, Placidi D, Gasparotti R, Pigozzi MG, Montani G, Alessio L (2000) Brain magnetic resonance imaging and manganese exposure. Neurotoxicology 21: 769-775

Maffeo E, Montuschi A, Stura G, Giordana MT (2014) Chronic acquired hepatocerebral degeneration, pallidal T1 MRI hyperintensity and manganese in a series of cirrhotic patients. Neurol Sci 35: 523-530

Martin CJ (2006) Manganese neurotoxicity: connecting the dots along the continuum of dysfunction. Neurotoxicology 27: 347-349

Meltzer HM, Brantsaeter AL, Borch-Iohnsen B, Ellingsen DG, Alexander J, Thomassen Y, Stigum H, Ydersbond TA (2010) Low iron stores are related to higher blood concentrations of manganese, cobalt and cadmium in non-smoking, Norwegian women in the HUNT 2 study. Environ Res 110: 497-504

Mergler D, Baldwin M, Belanger S, Larribe F, Beuter A, Bowler R, Panisset M, Edwards R, de GA, Sassine MP, Hudnell K (1999) Manganese neurotoxicity, a continuum of dysfunction: results from a community based study. Neurotoxicology 20: 327-342

Myers JE, Thompson ML, Naik I, Theodorou P, Esswein E, Tassell H, Daya A, Renton K, Spies A, Paicker J, Young T, Jeebhay M, Ramushu S, London L, Rees DJ (2003) The utility of biological monitoring for manganese in ferroalloy smelter workers in South Africa. Neurotoxicology 24: 875-883

Oulhote Y, Mergler D, Bouchard MF (2014) Sex- and age-differences in blood manganese levels in the U.S. general population: national health and nutrition examination survey 2011-2012. Environ Health 13: 87

Pan Y, Jackson RT (2008) Insights into the ethnic differences in serum ferritin between black and white US adult men. Am J Hum Biol 20: 406-416

Pesch B, Weiss T, Kendzia B, Henry J, Lehnert M, Lotz A, Heinze E, Kafferlein HU, Van Gelder R., Berges M, Hahn JU, Mattenklott M, Punkenburg E, Hartwig A, Bruning T (2012) Levels and predictors of airborne and internal exposure to manganese and iron among welders. J Expo Sci Environ Epidemiol 22: 291-298

Poniatowska R, Lusawa M, Skierczynska A, Makowicz G, Habrat B, Sienkiewicz-Jarosz H (2014) MRI brain findings in ephedrone encephalopathy associated with manganese abuse: Single-center perspective. Pol J Radiol 79: 150-155

Powell LW, Seckington RC, Deugnier Y (2016) Haemochromatosis. Lancet 388: 706-716

Rouault TA (2013) Iron metabolism in the CNS: implications for neurodegenerative diseases. Nat Rev Neurosci 14: 551-564

Sabati M, Maudsley AA (2013) Fast and high-resolution quantitative mapping of tissue water content with full brain coverage for clinically-driven studies. Magn Reson Imaging 31: 1752-1759

Wang W, Knovich MA, Coffman LG, Torti FM, Torti SV (2010) Serum ferritin: Past, present and future. Biochim Biophys Acta 1800: 760-769

Ward RJ, Zucca FA, Duyn JH, Crichton RR, Zecca L (2014) The role of iron in brain ageing and neurodegenerative disorders. Lancet Neurol 13: 1045-1060

Yokel RA (2006) Blood-brain barrier flux of aluminum, manganese, iron and other metals suspected to contribute to metal-induced neurodegeneration. J Alzheimers Dis 10: 223-253

Zecca L, Casella L, Albertini A, Bellei C, Zucca FA, Engelen M, Zadlo A, Szewczyk G, Zareba M, Sarna T (2008) Neuromelanin can protect against iron-mediated oxidative damage in system modeling iron overload of brain aging and Parkinson's disease. J Neurochem 106: 1866-1875

Zecca L, Stroppolo A, Gatti A, Tampellini D, Toscani M, Gallorini M, Giaveri G, Arosio P, Santambrogio P, Fariello RG, Karatekin E, Kleinman MH, Turro N, Hornykiewicz O, Zucca FA (2004a) The role of iron and copper molecules in the neuronal vulnerability of locus coeruleus and substantia nigra during aging. Proc Natl Acad Sci U S A 101: 9843-9848

Zecca L, Youdim MB, Riederer P, Connor JR, Crichton RR (2004b) Iron, brain ageing and neurodegenerative disorders. Nat Rev Neurosci 5: 863-873

Zucca FA, Segura-Aguilar J, Ferrari E, Munoz P, Paris I, Sulzer D, Sarna T, Casella L, Zecca L (2015) Interactions of iron, dopamine and neuromelanin pathways in brain aging and Parkinson's disease. Prog Neurobiol doi: 10.1016/j.pneurobio.2015.09.012

Table 1: Characteristics of the study groups of WELDOX II

	Total	Welders	Former welders	Controls	Parkinson disease	Hemochroma-tosis
	N=161	N=48	N=20	N=39	N=41	N=13

Age (years) [median, inter-quartile range]	56	50-65	51	47-56	65	57-69	54	50-65	60	56-68	56	55-67
Smoking status [n, %]												
Never	52	32.3%	10	20.8%	1	5.0%	16	41.0%	17	41.7%	8	61.5%
Former	62	38.5%	15	31.3%	11	55.0%	13	33.3%	18	43.9%	5	38.5%
Current	47	29.2%	23	47.9%	8	40.0%	10	25.6%	6	14.6%	0	0.0%
Blue collar worker as longest held occupation [n, %]	107	66.5%	48	100%	20	100%	16	41.0%	20	48.8%	3	23.1%
Gamma-glutamyl transferase ≥ 56 U/L [n, %]	29	18.0%	9	18.8%	7	35.0%	6	15.4%	6	14.6%	1	7.7%
Carbohydrate-deficient transferrin > 2.6% [n, %]	8	5.0%	4	8.3%	2	10.0%	2	5.1%	0	0.0%	0	0.0%

Table 2: Exposure to manganese and iron in the study groups of WELDOX II presented with median and inter-quartile range (all images)

		Total		**Welders**		**Former welders**		**Controls**		**Parkinson patients**		**Hemochromatosis**	
		N ≤ 161		**N ≤ 48**		**N ≤ 20**		**N ≤ 39**		**N ≤ 41**		**N ≤ 13**	
Manganese in blood (µg/L)		6.9	5.8-8.4	7.7	6.8-9.4	6.4	5.6-8.3	6.5	5.9-7.2	6.6	5.4-8.2	6.2	5.1-7.9
R1 (1/sec)	Globus pallidus	0.85	0.81-0.91	0.87	0.84-0.93	0.82	0.78-0.85	0.85	0.82-0.92	0.84	0.80-0.89	0.81	0.79-0.86

	Substantia nigra	0.76	0.71-0.80	0.76	0.72-0.80	0.79	0.72-0.83	0.76	0.72-0.80	0.78	0.70-0.82	0.73	0.69-0.77
	Frontal lobe	0.95	0.90-1.00	0.96	0.93-1.03	0.90	0.88-0.96	0.97	0.91-1.01	0.95	0.90-0.98	0.92	0.86-0.95
Free serum iron (µg/dL)		103	83-121	103	85-120	94	66-134	105	92-122	95	81-108	126	106-159
Serum ferritin (µg/L)		112	64-189	129	82-224	242	11-434	74	55-112	113	71-191	45	33-180
R2* (1/sec)	Globus pallidus	41	38-46	40	37-46	41	38-44	43	40-48	44	38-47	39	36-43
	Substantia nigra	45	39-51	42	39-47	45	41-56	45	40-51	49	40-56	43	40-53
	Frontal lobe	22	21-23	22	20-23	22	22-23	22	21-24	22	21-23	21	21-22

R1 and R2*: mean of both hemispheres

Table 3: Exposure to Mn and Fe in active and former welders by major process (median and inter-quartile range)

		Gas metal arc welding with solid and flux-cored wire				**Tungsten inert gas welding and other techniques**			
		Active N=22		**Former N=13**		**Active N=26**		**Former N=7**	
Respirable Mn (µg/m^3)		68	33-140	-	.-.	5	2-23	-	.-.
Manganese in blood (µg/L)		9.2	7.1-11.6	6.3	5.5-8.8	7.3	6.6-8.5	6.7	5.6-7.7
R1 (1/sec)	Globus pallidus	0.87	0.85-0.95	0.81	0.79-0.85	0.87	0.83-0.91	0.84	0.76-0.88

	Substantia nigra	0.76	0.73-0.80	0.74	0.71-0.81	0.76	0.71-0.78	0.80	0.77-0.84
	Frontal lobe	0.95	0.92-1.03	0.88	0.88-0.95	0.97	0.94-1.03	0.96	0.90-1.00
Respirable Fe (μg/m^3)		370	190-760	-	.-.	15	5-84	-	.-.
Free serum iron (μg/dL)		97	84-111	92	63-140	106	85-126	104	78-128
Serum ferritin (μg/L)		152	122-273	140	89-347	108	53-173	460	185-1080
R2* (1/sec)	Globus pallidus	40	36-44	42	38-43	40	37-47	41	38-45
	Substantia nigra	44	38-46	45	41-55	41	39-47	45	43-57
	Frontal lobe	22	20-23	22	22-23	22	21-24	22	21-23

Table 4: Spearman correlation coefficients with 95% confidence intervals between exposure variables in 161 men of WELDOX II (correlations with respirable Mn and Fe in 41 active welders)

r_s (95%CI)	MnB	R1 GP	R1 SN	R1 FL	AirFe	SFe	SF	R2* GP	R2* SN	R2* FL
Respirable Mn ($\mu g/m^3$)	0.10 (-0.19; 0.37)	0.05 (-0.25; 0.33)	0.16 (-0.13; 0.43)	-0.15 (-0.42; 0.15)	0.93 (0.88; 0.96)	0.14 (-0.15; 0.41)	0.32 (0.04; 0.56)	-0.11 (-0.38; 0.18)	-0.20 (-0.46; 0.09)	0.08 (-0.21; 0.36)
Mn in blood (µg/L) (MnB)		0.15 (-0.01; 0.31)	0.14 (-0.03; 0.29)	0.18 (0.02; 0.33)	0.12 (-0.17; 0.39)	-0.25 (-0.39; -0.10)	-0.12 (-0.27; 0.04)	-0.06 (-0.21; 0.10)	-0.14 (-0.29; 0.02)	0.09 (-0.06; 0.25)
R1 Global pallidus (GP)			0.32 (0.16; 0.46)	0.50 (0.37; 0.62)	0.00 (-0.29; 0.29)	0.02 (-0.15; 0.18)	-0.10 (-0.26; 0.07)	0.02 (-0.14; 0.18)	0.02 (-0.15; 0.18)	0.23 (0.07; 0.38)
R1 Substantia nigra (SN)				0.25 (0.09; 0.39)	0.15 (-0.14; 0.42)	-0.08 (-0.24; 0.09)	0.09 (-0.07; 0.25)	0.10 (-0.06; 0.26)	0.14 (-0.02; 0.30)	0.06 (-0.10; 0.22)
R1 Frontal lobe (FL)					-0.14 (-0.42; 0.15)	0.07 (-0.10; 0.23)	-0.14 (-0.30; 0.03)	0.06 (-0.11; 0.22)	-0.05 (-0.21; 0.12)	0.23 (0.07; 0.38)
Respirable Fe ($\mu g/m^3$) (AirFe)						0.06 (-0.22; 0.34)	0.36 (0.09; 0.59)	-0.11 (-0.38; 0.18)	-0.24 (-0.49; 0.05)	-0.03 (-0.31; 0.26)
Free serum Fe (µg/dL) (SFe)							0.14 (-0.01; 0.29)	0.08 (-0.08; 0.23)	0.04 (-0.11; 0.20)	0.11 (-0.05; 0.26)
Serum ferritin (µg/L) (SF)								0.08 (-0.08; 0.23)	-0.02 (-0.17; 0.14)	-0.08 (-0.23; 0.07)
R2* GP									0.61 (0.50; 0.70)	0.00 (-0.15; 0.15)
R2* SN										-0.02 (-0.18; 0.13)

Table 5: Potential predictors of the R1 and R2* relaxation rates in ROIs in 161 male participants of WELDOX II

Factor	**R1 (1/sec)**	**R2* (1/sec)**

	Globus pallidus		Substantia nigra		Frontal lobe		Globus pallidus		Substantia nigra		Frontal lobe	
	$\hat{\beta}$	P	$\hat{\beta}$	P	$\hat{\beta}$	P	$\hat{\beta}$	P	$\hat{\beta}$	P	$\hat{\beta}$	P
Intercept	0.881		0.684		1.041		16.7		17.4		19.2	
Controls (reference group)	0		0		0		0		0		0	
Active welders	0.011	0.600	-0.003	0.890	-0.016	0.370	-0.555	0.830	0.062	0.980	-0.510	0.260
Former welders	-0.046	0.080	-0.001	0.980	-0.031	0.150	-1.657	0.600	3.168	0.410	0.639	0.240
Parkinson patients	-0.009	0.670	0.014	0.470	-0.006	0.740	-1.030	0.690	4.252	0.180	0.609	0.170
Haemochromatosis patients	-0.014	0.610	-0.024	0.330	-0.042	0.070	-4.026	0.230	0.355	0.930	-0.304	0.600
Age [per 10 years]	-0.020	0.037	0.003	0.770	-0.029	<0.001	2.556	0.032	3.952	0.006	-0.245	0.240
Mn in blood [per 10 µg/L]	0.070	0.004	0.035	0.110	0.039	0.060	-1.851	0.540	-2.309	0.520	0.373	0.470
Serum ferritin [per 100 µg/L]	0.011	0.005	0.007	0.040	0.002	0.590	-0.288	0.540	-0.846	0.130	-0.075	0.340
R1 (1/sec)							17.1	0.030	12.6	0.210	4.441	0.018
R2* (1/sec)	0.001	0.025	0.000	0.210	0.002	0.200						
GGT [per 10 U/L]	-0.002	0.180	0.001	0.460	-0.001	0.440	0.097	0.590	0.017	0.940	-0.001	0.970
Left *versus* right hemisphere	-0.011	0.038	-0.005	0.270	0.031	<0.001	0.631	0.360	0.061	0.930	-0.119	0.590
Random effects												
Level-two variance estimate	0.006	<0.001	0.005	<0.001	0.004	<0.001	88.7	<0.001	134.6	<0.001	1.526	<0.001
Level-one variance estimate	0.002	<0.001	0.001	<0.001	0.002	<0.001	33.9	<0.001	34.6	<0.001	3.162	<0.001

Table 6: Potential predictors of the R1 and R2* relaxation rates in ROIs in welders of WELDOX II

a) Welding technique, Mn in blood and serum ferritin (active and former welders)

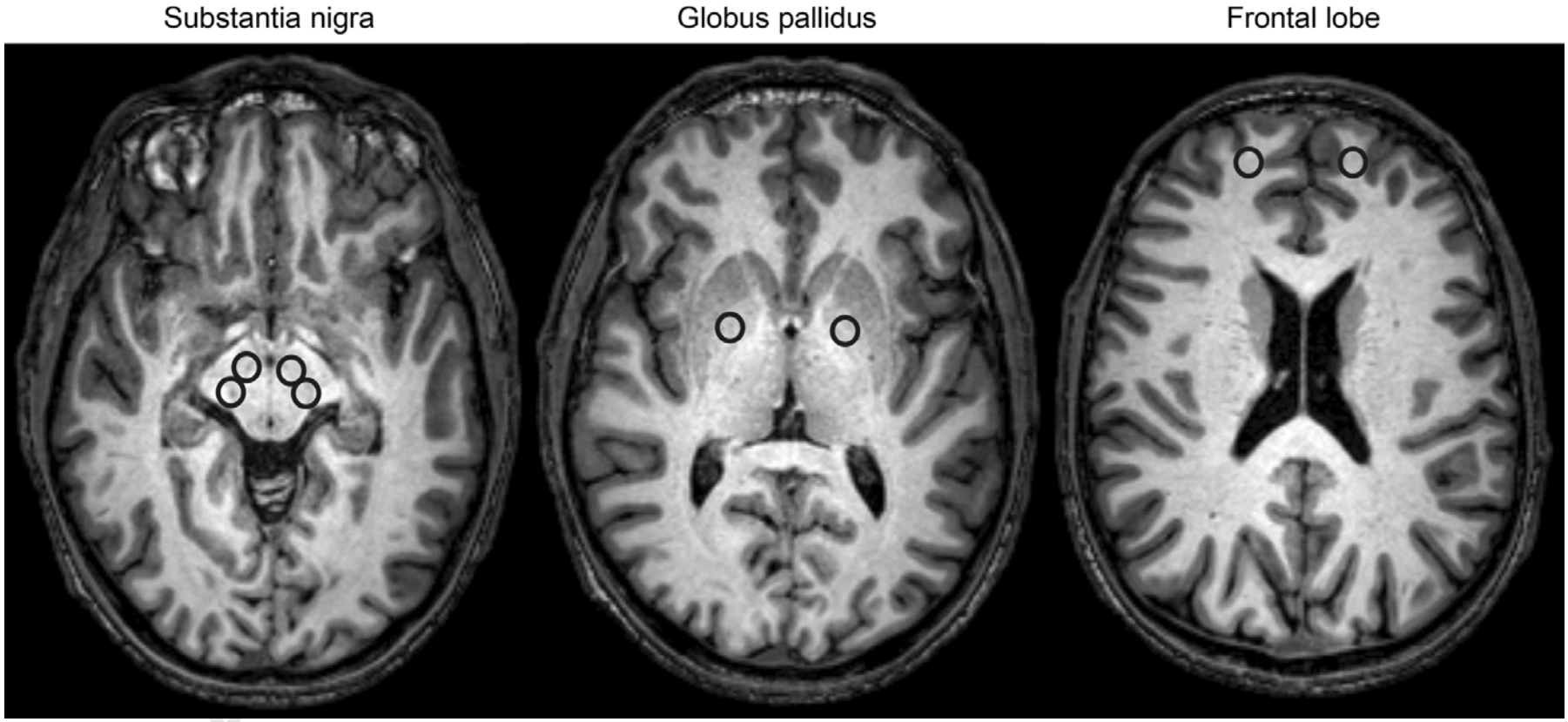
Factor	R1 (1/sec)						R2* (1/sec)					
	Globus pallidus		Substantia nigra		Frontal lobe		Globus pallidus		Substantia nigra		Frontal lobe	
	$\hat{\beta}$	P	$\hat{\beta}$	P	$\hat{\beta}$	P	$\hat{\beta}$	P	$\hat{\beta}$	P	$\hat{\beta}$	P
Intercept	0.891		0.673		0.999		13.0		11.8		14.1	
TIG or other techniques	0		0		0		0		0		0	
GMAW or FCAW	0.016	0.51	0.002	0.91	-0.025	0.16	-1.736	0.57	-0.045	0.99	-0.125	0.77
Mn in blood [per 10 µg/L]	0.113	<0.001	0.039	0.13	0.050	0.03	-2.695	0.51	-0.146	0.97	-0.360	0.53
Serum ferritin [per 100 µg/L]	0.013	0.01	0.008	0.041	0.003	0.39	-0.445	0.45	-0.605	0.28	-0.079	0.34
R1 (1/sec)	-	-	-	-	-	-	26.9	0.021	10.5	0.45	7.198	0.01
R2* (1/sec)	0.002	0.017	0.000	0.55	0.003	0.11	-	-	-	-	-	-
Age [per 10 years]	-0.037	0.023	0.004	0.76	-0.031	0.007	1.670	0.39	4.787	0.011	0.298	0.29
GGT [per 10 U/L]	-0.003	0.19	0.001	0.48	-0.000	0.72	0.231	0.32	0.186	0.40	-0.017	0.61
Left *versus* right hemisphere	-0.009	0.26	-0.006	0.33	0.031	<0.001	1.233	0.28	0.437	0.68	0.086	0.80
Random effects												
Level-two variance estimate	0.009	<0.001	0.006	<0.001	0.004	<0.001	126.9	<0.001	116.9	<0.001	1.131	0.036
Level-one variance estimate	0.002	<0.001	0.001	<0.001	0.001	<0.001	42.4	<0.001	38.1	<0.001	3.666	<0.001

b) Respirable Mn or Fe (active welders)

Factor	R1 (1/sec)						R2* (1/sec)					
	Globus pallidus		Substantia nigra		Frontal lobe		Globus pallidus		Substantia nigra		Frontal lobe	
	$\hat{\beta}$	P	$\hat{\beta}$	P	$\hat{\beta}$	P	$\hat{\beta}$	P	$\hat{\beta}$	P	$\hat{\beta}$	P

Intercept		0.674		0.652		1.056		26.4		19.2		16.3	
Respirable Mn	< 20 μg/m^3	0		0		0							
	20 – 100 μg/m^3	0.017	0.68	-0.009	0.76	-0.027	0.31	-	-	-	-	-	-
	>100 μg/m^3	0.123	0.01	0.049	0.16	-0.009	0.78	-	-	-	-	-	-
Respirable Fe	< 25 μg/m^3							0		0		0	
	25-310 μg/m^3	-	-	-	-	-	-	-2.752	0.54	0.440	0.88	-0.354	0.60
	> 310 μg/m^3	-	-	-	-	-	-	-7.095	0.14	-5.563	0.08	0.123	0.86
R1 (1/sec)		-	-	-	-	-	-	37.3	0.004	23.2	0.07	6.375	0.07
R2* (1/sec)		0.003	<0.001	0.001	0.12	0.001	0.64	-	-	-	-	-	-
Age [per 10 years]		0.017	0.60	0.009	0.71	-0.022	0.31	-2.929	0.41	1.771	0.46	-0.101	0.85
GGT [per 10 U/L]		-0.002	0.36	0.002	0.30	-0.000	0.99	0.003	0.99	-0.177	0.33	-0.031	0.45
Left *versus* right hemisphere		-0.007	0.43	-0.013	0.08	0.029	<0.001	2.204	0.08	0.298	0.81	0.327	0.46
Random effects													
Level-two variance estimate		0.013	<0.001	0.007	<0.001	0.005	<0.001	130.9	<0.001	50.0	<0.001	1.189	0.08
Level-one variance estimate		0.002	<0.001	0.001	<0.001	0.001	<0.001	35.7	<0.001	34.1	<0.001	4.201	<0.001

Figure 1: Regions of interest including the globus pallidus, substantia nigra and the white matter of the frontal lobe


Substantia nigra
Globus pallidus
Frontal lobe